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**ACUTE MOUNTAIN SICKNESS:
RELATIONSHIP TO BRAIN VOLUME AND
EFFECT OF ORAL GLYCEROL PROPHYLAXIS**

**U S ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts**

JUNE 1998



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13. ABSTRACT (Maximum 200 words) The objective of this study was to test the hypothesis that oral glycerol administration prior to and during exposure to high altitude will decrease the prevalence and severity of AMS. The basis for this hypothesis was that glycerol would create an osmotic gradient between the blood and the cerebral spinal fluid producing a net movement of water out of the CNS, thus attenuating development of cerebral edema. Furthermore, to assess whether AMS was associated with development of cerebral edema, we used MR imaging and post-processing to quantify changes in brain tissue volume, hypothesized to increase due to cerebral edema. MRI studies were performed in subjects at sea level and following a 32-h exposure to an altitude of 4,572 m in a hypobaric chamber. The study found that oral administration of glycerol did not alleviate the symptoms associated with AMS, and that whole brain volume was increased by ~2% following the hypobaric exposure. There was no relationship between the AMS prevalence or severity and the magnitude of the brain volume changes.				
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Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRMC Regulation 70-25 on the use of volunteers in research.

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ACUTE MOUNTAIN SICKNESS:
RELATIONSHIP TO BRAIN VOLUME AND
EFFECT OF ORAL GLYCEROL PROPHYLAXIS

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BACKGROUND

Mountain environments are likely areas of military confrontation. Mountain ranges typically form the borders of nations, and numerous regions of geopolitical interest to the U.S. such as the Balkans, South America, the Middle East, and Asia contain extensive areas of moderate (>1500 m) to high (>2400 m) altitudes. Rapid force projection to such altitudes presents challenges in sustaining optimal military performance due to the hypoxia associated with altitude exposure and its deleterious affect on mission-related work activities. Acute Mountain Sickness (AMS), caused by hypobaric hypoxia, will negatively impact military operations. Current interventions to prevent AMS include slow or staged ascent and select pharmaceuticals. However, each currently available procedure or medication for prophylaxis or treatment of AMS can constrain or degrade mission effectiveness independent of AMS. Thus, there is a need to evaluate promising pharmacological and nutritional interventions which may prevent or minimize the effects of AMS.

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EXECUTIVE SUMMARY

Acute Mountain Sickness (AMS) is a multi-system disorder which is principally characterized by headache, anorexia, nausea, vomiting, insomnia, lassitude, and malaise. The syndrome is common in unacclimatized low altitude residents who rapidly ascend to terrestrial elevations exceeding 2,500 m. The symptoms usually appear within 24 h of exposure and normally resolve after several days. Acute Mountain Sickness is usually self-limited, but may progress into high altitude cerebral edema (HACE) or high altitude pulmonary edema (HAPE), both of which are life-threatening. The most widely accepted hypothesis of the etiology of AMS is that the symptoms are a manifestation of hypoxia-induced, "subclinical" cerebral edema that causes swelling of the brain. However, the role of cerebral edema in AMS has remained hypothetical because its presence has not been adequately documented in individuals with AMS symptoms.

Currently, acetazolamide is the only FDA approved pharmaceutical prophylaxis for AMS. It is effective in preventing AMS by increasing ventilation and promoting diuresis, thus, facilitating acclimatization. However, the medication can cause various adverse effects which can degrade mission performance.

Osmotic agents are potential alternatives to acetazolamide for prophylaxis of AMS. One such osmotic agent, glycerol, is particularly attractive because it can be ingested, is rapidly absorbed and metabolized, and is nontoxic. Glycerol increases plasma osmolality. It is evenly distributed to the body tissues, although it enters the central nervous system (CNS) very slowly. This causes an osmotic gradient between the blood and the cerebral spinal fluid which results in a net movement of water out of the CNS. Therefore, the action of exogenous glycerol has the potential to attenuate cerebral edema and thereby decrease AMS symptoms.

The objective of this study was to test the hypothesis that oral glycerol administration prior to and during exposure to high altitude would decrease the prevalence and severity of AMS. Additionally, to assess whether AMS was associated with development of cerebral edema, we used a magnetic resonance imaging (MRI) and post-

processing technique to quantify changes in brain tissue volume, which we hypothesized to increase in the presence of cerebral edema.

A double-blind, placebo-controlled, cross-over design was used to test the effect of oral glycerol on prevention of AMS. Eleven male volunteers were exposed to a simulated altitude of 4572 m ($P_B = 430$ torr) in a hypobaric chamber for ~32 h on 2 occasions, once while consuming 1.0 g/kg body weight glycerol in 300 ml of orange juice every 8 h and once while consuming a placebo solution. The AMS symptoms were assessed using the Environmental Symptoms Questionnaire. After ~32 h hypobaric exposure, brain volume was measured by 3-dimensional segmentation of volume MRI data.

None of the volunteers were able to consume the full dose of glycerol due to a combination of the severe gastrointestinal distress associated with AMS and the unpalatability of the glycerol solutions. Within 12 h of altitude exposure, all volunteers who developed AMS were symptomatic in both the placebo and glycerol trials. In comparison to the placebo trial, glycerol treatment increased AMS prevalence and severity, but not significantly so. The time course for development of AMS was similar between the treatment and placebo trials.

Following ~32 h hypobaric exposure, whole brain volume significantly increased by ~2.2% in all subjects exposed to high altitude. Glycerol consumption had no effect on the magnitude of the brain volume increase. The magnitude of the brain volume increase was inversely related to the resting arterial oxygen saturation, but the relationship was not statistically significant. However, there was no significant relationship between the presence or severity of AMS and the magnitude of the brain volume increase. The increase in brain volume was almost entirely within the gray matter.

In summary, this study found that oral glycerol consumption did not decrease the prevalence or severity of AMS in young healthy men. Rather, ingesting the glycerol solution either produced symptoms mimicking AMS or accentuated the AMS symptoms. Following the 32 h of high altitude exposure, MRI results revealed a consistent and reproducible increase in brain volume, primarily of the gray matter, consistent with

development of diffuse cerebral edema. However, there was no apparent relationship between the AMS prevalence or severity and the magnitude of the brain volume changes.

INTRODUCTION

Acute Mountain Sickness (AMS) is a syndrome that is characterized by headache, anorexia, nausea, vomiting, insomnia, lassitude, and malaise. The syndrome has great individual variation in susceptibility; however, the hypoxia-induced symptoms are most common in unacclimatized low altitude residents who rapidly ascend to terrestrial elevations exceeding 2,500 m (Johnson and Rock, 1988). The symptoms of AMS commonly appear within 4 to 24 h of exposure, and usually resolve after several days as acclimatization to hypoxia is achieved. Acute Mountain Sickness is usually self-limited, but may progress into high altitude cerebral edema (HACE) or high altitude pulmonary edema (HAPE), both of which are potentially life-threatening.

ETIOLOGY OF AMS

Although there has been much speculation about the cause of AMS symptoms, little definitive information exists. The most widely accepted hypothesis is that the symptoms are a manifestation of hypoxia-induced, "subclinical" cerebral edema that causes swelling of the brain (Sutton and Lassen, 1979; Singh, Khanna, Srivastava, Lal, Roy, and Subramanyam, 1969; Hansen and Evans, 1970). However, the role of cerebral edema has remained hypothetical because its presence has not been adequately documented in individuals with symptoms of AMS. Given the usually benign nature of AMS, attempts to document concomitant cerebral edema have used only non-invasive imaging. Thus, Levine and colleagues (Levine, Yoshimura, Kobayashi, Fukushima, Shibamoto, and Ueda, 1989) reported a decrease in white matter density of the brain consistent with cerebral edema on Computerized Tomography (CT) scans in one out of six symptomatic individuals during two 48-hr exposures to an altitude-equivalent pressure of 3700 m in a hypobaric chamber. Similarly, Matsuzawa and colleagues (Matsuzawa et al., 1992) found "mildly increased signal intensity of the white matter consistent with vasogenic cerebral edema" on magnetic resonance imaging (MRI) scans of the brain in four of seven individuals with AMS symptoms following a 24-hour exposure to 3700 m in a hypobaric chamber. While findings consistent with cerebral edema in some of the subjects with AMS in these two studies are supportive of a causative role, the lack of evidence for edema in more than half of the subjects with AMS leaves the hypothesis in doubt. However, the lack

of findings may be the result of insensitive technique rather than an incorrect hypothesis. Both studies relied entirely upon clinical interpretation of the images by panels of radiologists who may not have been able to detect very low ("subclinical") levels of brain edema using subjective interpretation of tissue density changes.

PHARMACOLOGICAL PROPHYLAXIS OF AMS

Currently, the only FDA approved pharmacological prophylaxis of AMS is acetazolamide. Acetazolamide is effective in preventing AMS primarily by increasing minute ventilation, improving arterial oxygenation, promoting diuresis, and thus facilitating acclimatization (Hackett, Schoene, Winslow, Peters, and West,1985). However, acetazolamide can cause various adverse effects which include gastric distress, nausea, vomiting, diarrhea, constipation, anorexia, weight loss, malaise, fatigue, headache, weakness and paresthesia (Johnson and Rock,1988; Hultgren,1992). More debilitating, but less common, reactions include confusion, ataxia, tremor, myopia, thrombocytopenia, leukopenia, and aplastic anemia (Johnson and Rock,1988; Hultgren,1992).

The synthetic corticosteroid, dexamethasone, has also been administered to prevent and treat AMS (Rock, Johnson, Larsen, Fulco, Trad, and Cymerman,1989; Rock, Johnson, Cymerman, Burse, Falk, and Fulco,1987; Johnson, Rock, Fulco, Trad, Spark, and Maher,1984; Hackett, Roach, Wood, Foutch, Meehan, Rennie et al.,1988). The primary concern regarding dexamethasone use is that acclimatization appears to be disrupted, and AMS tends to develop upon cessation of the drug (Rock, Johnson, Larsen, Fulco, Trad, and Cymerman,1989; Rock, Johnson, Cymerman, Burse, Falk, and Fulco,1987; Hackett, Roach, Wood, Foutch, Meehan, Rennie et al.,1988). As a result, the drug intervention must be continued throughout the exposure to avoid reoccurrence of AMS. Depression, hyperglycemia, and gastrointestinal bleeding, which are all serious conditions, have been associated with dexamethasone treatment (1). Even more importantly, this steroid can depress the immune response, which would be extremely detrimental in a combat environment (Rock, Johnson, Larsen, Fulco, Trad, and Cymerman,1989). Consequently, dexamethasone is not currently recommended as a prophylaxis for AMS (Rock, Johnson, Cymerman, Burse, Falk, and Fulco,1987; Rock, Johnson, Larsen, Fulco, Trad, and Cymerman,1989; Hackett, Roach, Wood, Foutch, Meehan, Rennie et al.,1988).

Strong loop diuretics, such as furosemide and bumetanide have also been reported to be effective in the treatment of HACE and severe cases of AMS (Singh, Khanna, Srivastava, Lal, Roy, and Subramanyam,1969; Hackett et al.,1989). However, the severe polyuria which accompanies the administration of furosemide has been reported to cause hypovolemia and hemoconcentration, which subsequently results in hypotension, syncope, alkalosis, ataxia and electrolyte imbalances (Hultgren,1992; Gray, Bryan, Frayser, Houston, and Rennie,1971).

Utilization of osmotic agents, such as glycerol and mannitol, for treatment of high altitude illnesses has been briefly mentioned in the literature (Hackett et al.,1989); however, to our knowledge, no studies have been conducted. The characteristics of glycerol make it an attractive, potential alternative to the other drug therapies for treatment and prevention of AMS (Tourtellotte, Reinglass, and Newkirk,1972; Frank, Nahata, Milo, and Hilty,1981). These characteristics include its osmotic action, rapid absorption, metabolism, and non-toxicity. Glycerol is rapidly and evenly distributed to the body tissues, yet, it does not readily cross the blood-brain barrier into the central nervous system (CNS) (Waterhouse and Coxon,1970). Glycerol enters the brain, cerebrospinal fluid (CSF), and aqueous humor of the eyes very slowly (Cantore, Guidetti, and Virno,1964). Glycerol ingestion can cause an increase in plasma osmolality (McCurdy, Schneider, and Schele,1966). The high plasma osmolality results in an osmotic gradient between the blood and the CSF, which in turn, causes a net movement of water out of the CNS. Therefore, it seems that ingested glycerol has the potential to prevent or lessen AMS.

Oral doses of glycerol have been used to decrease the high intraocular pressure associated with glaucoma (McCurdy, Schneider, and Schele,1966; Jaffe and Light,1965). Oral doses of glycerol (1 to 1.27 g/kg) significantly reduced intraocular pressure within 7 to 12 min after ingestion (McCurdy, Schneider, and Schele,1966). That study also demonstrated that the maximal CNS dehydration occurred at 60 to 90 min, which corresponded to a serum glycerol level of 100 mg/dl. Glycerol ingestion has been demonstrated to reduce intracranial hypertension in cases of cerebral edema (Tourtellotte, Reinglass, and Newkirk,1972; Cantore, Guidetti, and Virno,1964). Cantore et al. (Cantore, Guidetti, and Virno,1964) reported that glycerol doses of 0.5 to 2.0 g/kg were effective in alleviating cerebral edema without toxic consequences. Other investigations have also

demonstrated that CSF volume and pressure can be reduced with oral ingestion of 0.5 to 1.5 g/kg glycerol (Tourtellotte, Reinglass, and Newkirk,1972; Rottenberg, Hurwitz, and Posner,1977).

Whereas the previous discussion of the clinical utilization of exogenous glycerol tends to support its high potential for attenuating AMS, there is some possibility that oral glycerol could exacerbate AMS. Although glycerol ingestion has not been shown to have any physiological effects which would interfere with normal altitude acclimatization, ingesting glycerol containing solutions may produce nausea and headache in a small portion of the population (Tourtellotte, Reinglass, and Newkirk,1972; Murray, Eddy, Paul, Seifert, and Halaby,1991). In those individuals the adverse reactions to glycerol ingestion could accentuate or mimic the AMS symptoms.

OBJECTIVE

The objective of this study was to test the hypothesis that glycerol ingestion prior to and during exposure to high altitude would decrease the prevalence and severity of AMS. The basis for this hypothesis was that the osmotic activity of glycerol would create an osmotic gradient between the blood and the cerebral spinal fluid, producing a net movement of water out of the CNS, thereby preventing development of cerebral edema. Furthermore, to assess whether AMS was associated with development of cerebral edema, we used a technique of 3-dimensional segmentation of volume magnetic resonance imaging data (Kapur et al.,1995; Wells, Grimson, Kikinis, and Jolesz,1996; Matsumae, Kikinis, Morocz, Lorenzo, Sandor, Albert et al.,1996; Matsumae, Kikinis, Morocz, Lorenzo, Albert, Black et al.,1996) to quantify changes in brain tissue volume, which we hypothesized would increase in the presence of cerebral edema.

METHODS

SUBJECTS

Eleven male military volunteers with a mean (\pm SD) age and body weight of 21 ± 3 yrs and 73 ± 10 kg participated in this study. Each was a lifelong low altitude resident and had no exposure to altitudes greater than 1000 m for at least 6 months immediately

preceding the study. All volunteers received medical examinations, and none were found to have any condition that would warrant exclusion from the study. All participated in regular military physical training and were of average fitness. Each gave written and verbal acknowledgment of his informed consent and was made aware of his right to withdraw without prejudice at any time.

PROTOCOL

A double-blind, placebo-controlled, cross-over design was used. Volunteers were exposed to a simulated altitude of 4572 m ($P_B = 430$ torr) in a hypobaric chamber for approximately 32 h on two occasions: once while consuming 1.0 g/kg body weight glycerol (Penta Manufacturing, Fairfield, NY) in 300 ml of orange juice immediately before decompression to altitude and every 8 h thereafter, and once while consuming a placebo solution (300 ml orange juice and Nutrasweet). The order of glycerol administration was counterbalanced, and at least 14 days elapsed between exposures.

For each trial, volunteers first spent 48 h in the hypobaric chamber at sea level undergoing baseline measurements. During this baseline period, the subjects underwent a volume MR imaging protocol to measure their brain volume. On the morning of the third day, the chamber was decompressed to 430 torr over 15 min. Food and fluid consumption was *ad libitum* throughout the study except for one 8-h period in the early morning to facilitate body fluid compartment measurements. After ~32 h the chamber was recompressed to sea level, and the subjects removed to conduct the brain volume MR imaging and measurements. Immediately upon recompression to sea level, each volunteer breathed a hypoxic gas mixture ($FIO_2 = 0.12$) through non-rebreathing face masks in order to maintain his arterial oxygen saturation at 4300 m levels during the MRI studies.

MEASUREMENTS

AMS symptoms were assessed utilizing the Environmental Symptoms Questionnaire (ESQ), which was administered at 0600 h and 2000 h during sea level testing and at 0400, 1200 h, and 2000 h at high altitude. The ESQ is a self-reported 67-question symptom inventory designed to quantify symptoms induced by altitude and other stressful environments and conditions (Sampson, Cymerman, Burse, Maher, and Rock, 1983). To document the presence of AMS, a weighted average of cerebral (AMS-C) and respiratory

(AMS-R) symptoms were calculated from the ESQ scores (Sampson, Cymerman, Burse, Maher, and Rock, 1983). An AMS-C value of 0.7 or greater or an AMS-R value greater than 0.6 indicates the presence of AMS. The effectiveness of AMS-C scores in identifying individuals with AMS has been previously reported and validated (Sampson, Cymerman, Burse, Maher, and Rock, 1983). To compare AMS severity between trials, each subject's AMS-C and AMS-R score was converted to an AMS severity index ranging between 0-8 (Sampson, Cymerman, Burse, Maher, and Rock, 1983).

To assess the degree of hypoxic stress among the volunteers, arterial oxygen saturation was measured by finger pulse oximetry (SpO_2) (SensorMedics Corp, Oxyshuttle) intermittently throughout the altitude exposure.

To document serum glycerol level and its osmotic effect, venous blood samples were obtained by aseptic venipuncture of an arm vein and analyzed in triplicate for serum glycerol concentration (Monarch Chemistry System Model 761, Instrumentation Laboratory) and blood osmolality (Advanced Micro-osmometer, Advanced instruments) at regular intervals.

The 3-dimensional MRI data sets for the calculations of brain tissue volumes were collected using a 1.5 T Signa Scanner (GE, Milwaukee, WI). In order to ensure immobilization during the scan and precise repositioning of the head between scans, a hard urethane foam head mold, specific for each subject, was fitted into a cylindrical plastic chamber within a quadrature radio frequency head coil (GE Medical Systems, Milwaukee, WI). A hypoxic gas mixture ($\text{FIO}_2=0.12$) was pumped through the head coil chamber to maintain each subject's arterial oxygen saturation at 4300 m levels during the MRI studies. The MRI protocol consisted of three imaging series: (1) A T1-weighted spin echo sagittal series (TE 19 ms, TR 600 ms, 24 cm FOV, 4 mm thick, 1 mm spaced 256X192 matrix, 1 NEX, 24 slices) was used as a localizer followed by, (2) dual echo fast spin echo axial series (TE1 30 ms, TE2 80 ms, TR 3000 ms, 24 cm FOV, 3 mm thick, 256X192 matrix, 0.5 NEX, 52 slices) for T2-weighted and proton density-weighted images and (3) a spoiled GRASS (gradient-recalled echo) coronal volumetric series (FA 45 deg, TE 5 ms, TR 35 ms, 24 cm FOV, 1.5 mm thick, 256X192 matrix, 1 NEX, 124 slices). Segmentation of the coronal image data into white and gray matter volumes was performed on a PVS supercomputer (Power Visualization system, IBM, Hathorn, NJ) with a method of adaptive

3D segmentation that uses knowledge of tissue properties and corrects for RF deposition inhomogeneities (Kapur et al., 1995; Wells, Grimson, Kikinis, and Jolesz, 1996; Matsumae, Kikinis, Morocz, Lorenzo, Sandor, Albert et al., 1996; Matsumae, Kikinis, Morocz, Lorenzo, Albert, Black et al., 1996). The scan data from each volunteer were subjected to the fully automated computer segmentation using the same post-processing parameters. Balloon phantom experiments demonstrated that the technique was highly sensitive and capable of detecting relative volume changes within the range that occurred in the human brain (± 20 ml or $\pm 1.66\%$). In addition, three repeated volume calculations of the same control brain on three separate occasions were reproducible within $\pm 0.5\%$ of each other.

STATISTICAL ANALYSIS

Values are presented as mean \pm standard deviation (SD) except where noted. Data were analyzed using standard two-way repeated-measures analysis of variance. Significant main effects were localized using Tukey's least significant difference post hoc test. Tests of possible relationships between variables were performed using the Pearson Product-Moment Correlation method. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Eleven volunteers started the first altitude exposure trial (Table 1). Two voluntarily withdrew prior to completing the 32 h altitude exposure due to severe AMS symptoms. Eight of the 11 volunteers participated in the second altitude exposure trial. One of these 8 withdrew after ~12 h of altitude exposure due to severe symptoms of AMS.

TABLE 1. Subject treatment order and altitude illness outcome.

SUBJECT	TRIAL 1		TRIAL 2	
	TREATMENT	AMS	TREATMENT	AMS
1	Glycerol	Yes*	WD	-
2	Glycerol	Yes	Placebo	No
3	Glycerol	Yes	Placebo	No
4	Placebo	Yes	Glycerol	Yes
5	Glycerol	Yes	WD	-
6	Placebo	No	Glycerol	No
7	Glycerol	Yes	Placebo	Yes
8	Placebo	Yes	Glycerol	Yes
10	Glycerol	Yes	Placebo	No
11	Placebo	Yes*	Glycerol	Yes*
12	Glycerol	Yes	WD	-

*removed from hypobaric exposure due to severe symptoms of AMS

WD: withdrew from study prior to second trial

During the placebo trial, 4 of the 8 volunteers developed AMS while 10 of the 11 volunteers developed AMS during the glycerol trial (Table 1). The AMS prevalence and severity is illustrated in Figure 1 for the 8 subjects who participated in both trials. Within 12 h of altitude exposure, all volunteers who were to develop AMS were symptomatic in both the placebo and glycerol trials. The AMS symptom severity peaked between 14-22 h of altitude exposure and AMS prevalence and symptom scores tended ($p=0.11$) to be greater during the glycerol trial compared to the placebo trial.

The group's SpO_2 during the first 10 hours of altitude exposure was similar ($78 \pm 5\%$ and $75 \pm 11\%$) during the placebo and glycerol trials, respectively. As expected, there was a large inter-subject variation in the resting SpO_2 . However, each volunteer's SpO_2 was

highly correlated ($r=0.88$, $p<0.01$) between trials (Figure 2), suggesting that the hypobaric hypoxic stimulus to develop AMS was consistent between trials.

None of the volunteers was able to consume the targeted dose of glycerol due to a combination of the severe gastrointestinal distress associated with AMS and the unpalatability of the glycerol solutions. Consequently, the plasma glycerol concentration (Figure 3) was highly variable and did not approach the estimated clinically effective plasma concentration (~100 mg/dl). Therefore, the blood osmolality in the glycerol treatment trial was not significantly different from the placebo trial (Figure 3).

TABLE 2. Whole Brain volume at SL and following HA exposure.

SUBJECT	PLACEBO TRIAL		GLYCEROL TRIAL	
	SL	HA	SL	HA
1	WD	WD	WD	WD
2	1435.9	1464.7	1457.6	1451.6
3	1217.9	1250.3	1222.8	1248.2
4	1272.8	1297.9	1264.8	1285.7
5	WD	WD	1356.9	1388.4
6	1271.5	1285.7	1240.6	1243.1
7	1339.5	1375.2	1336.8	1396.7
8	1205.5	1230.1	1199.0	1230.0
10	1271.0	1292.8	1237.2	1308.5
11	WD	WD	1222.9	1248.2
12	WD	WD	1240.6	1278.1

WD: withdrew from study

Brain volume measurements were successfully completed on 7 and 10 subjects in the placebo and glycerol trials, respectively (Table 2). For the 7 subjects who successfully

completed both trials, the SL brain volume measurements differed by less than 0.5% (1287.7 ± 78.6 ml vs 1279.8 ± 89.6 ml) between the placebo and glycerol trials, respectively. After 32-h at high altitude, all individuals experienced an expansion of brain volume during the placebo trial and 6 of 7 experienced expansion during the glycerol trial. Figure 4 illustrates the percentage increases in whole brain, gray matter and white matter volumes following high altitude exposure. Whole brain volume was increased ($p < 0.001$) at high altitude above sea level values by 26.1 ± 7.1 ml and 29.3 ± 28.1 ml for the placebo and glycerol trials, respectively. There was no significant treatment effect on brain volume expansion. Expansion of brain volume was primarily due to a increase in the gray matter volume ($p < 0.05$), with very little change ($< 1.0\%$) in the white matter volume (Figure 4).

Due to the possibility that glycerol influenced the presence and severity of AMS symptoms, evaluation of correlations between physiological variables and AMS was limited to the placebo trial. During the placebo trial, the magnitude of the brain volume increase tended ($p = 0.1$) to be greater in subjects showing the greatest degree of hypoxemia as measured by SpO_2 (Figure 5). As illustrated in Figure 6, during the placebo trial similar increases in brain volume were found in subjects with and without AMS. Also, no significant correlation was found between each subject's highest ESQ-C score and change in brain volume during the placebo trial (Figure 7).

FIGURE 1
AMS prevalence and severity plotted as a function of duration at altitude in the glycerol and placebo trials

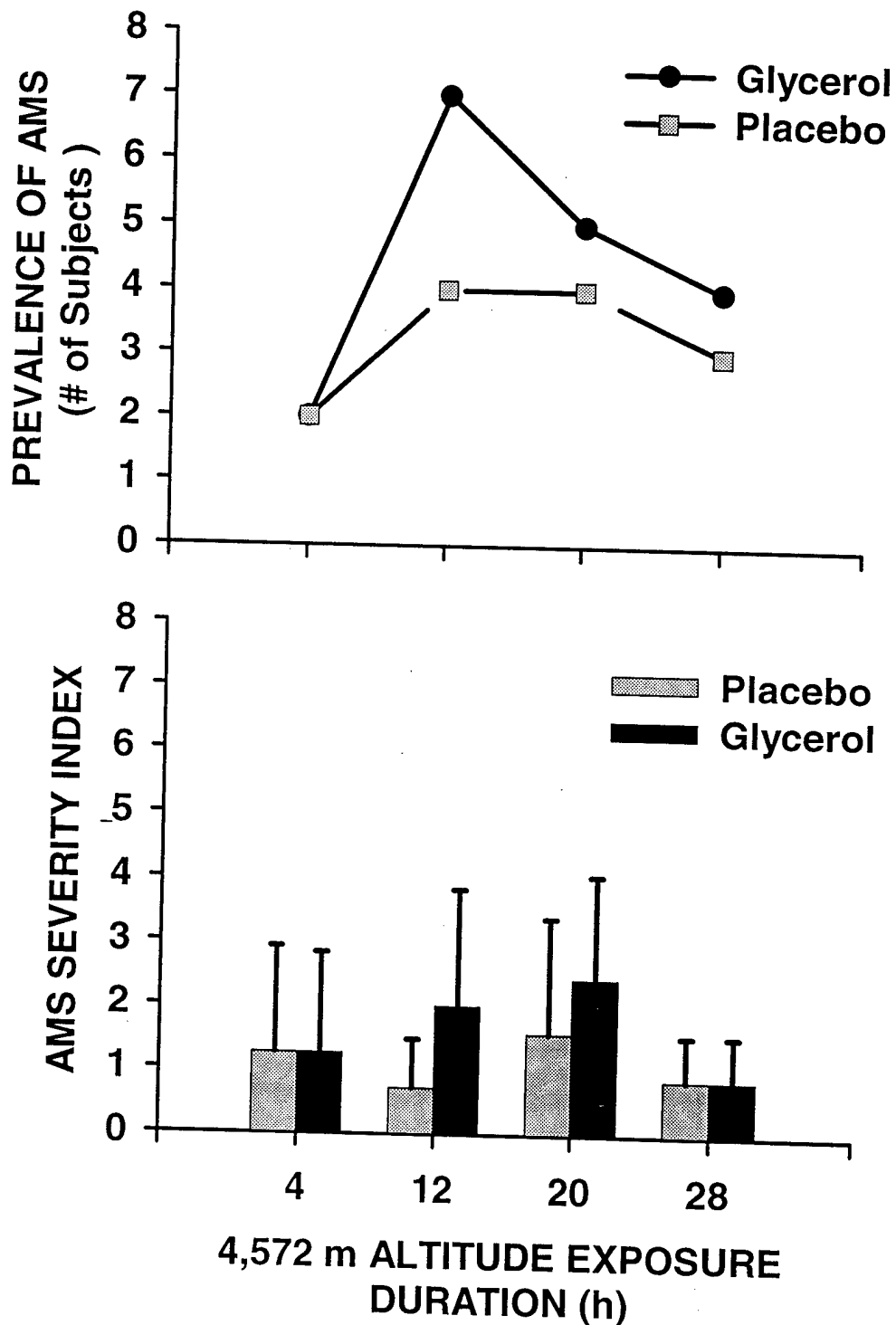


FIGURE 2
Within subject resting SpO₂
between placebo and glycerol trials

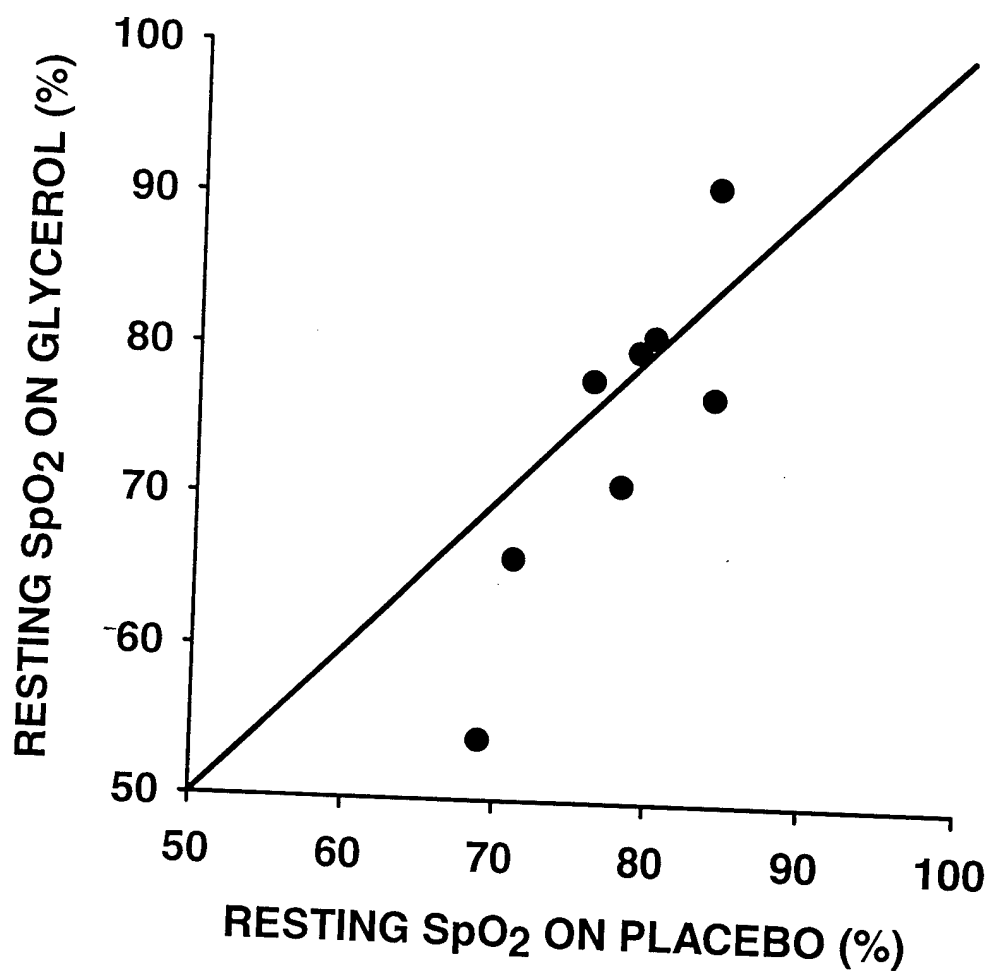


FIGURE 3
 Serum glycerol and blood osmolality concentrations
 relative to target level

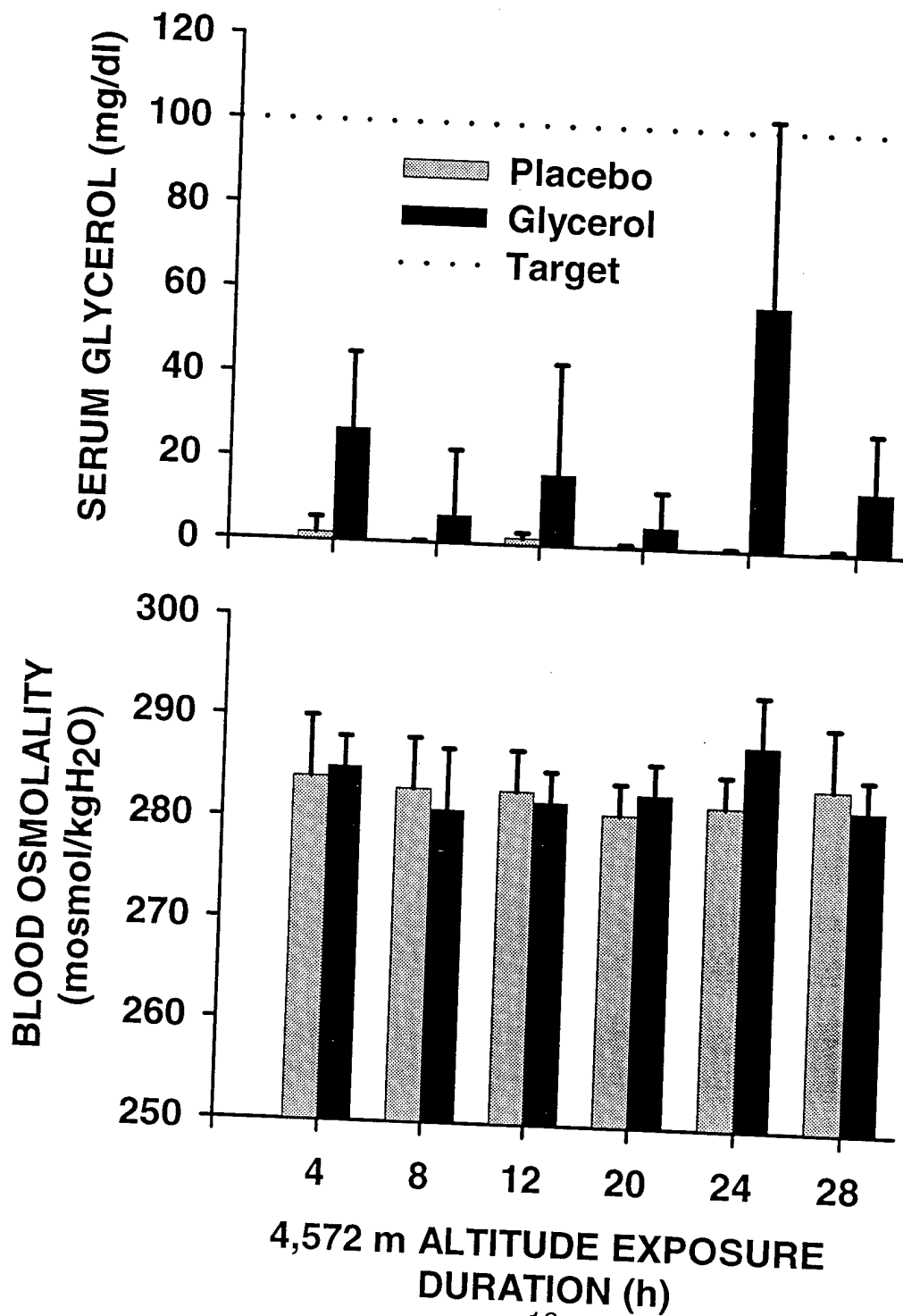


FIGURE 4
Effect of 30 h hypobaric-hypoxic exposure
on brain volume

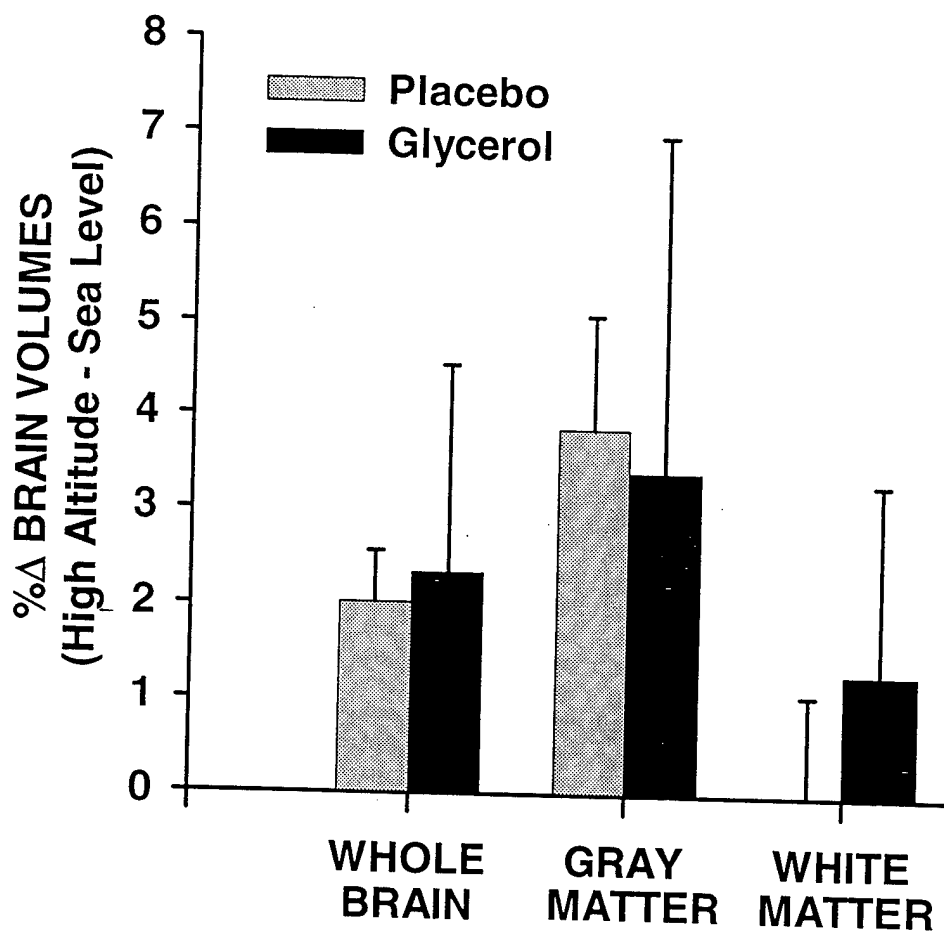


FIGURE 5
The magnitude of the brain volume increase plotted as a function of the degree of hypoxemia (placebo trial)

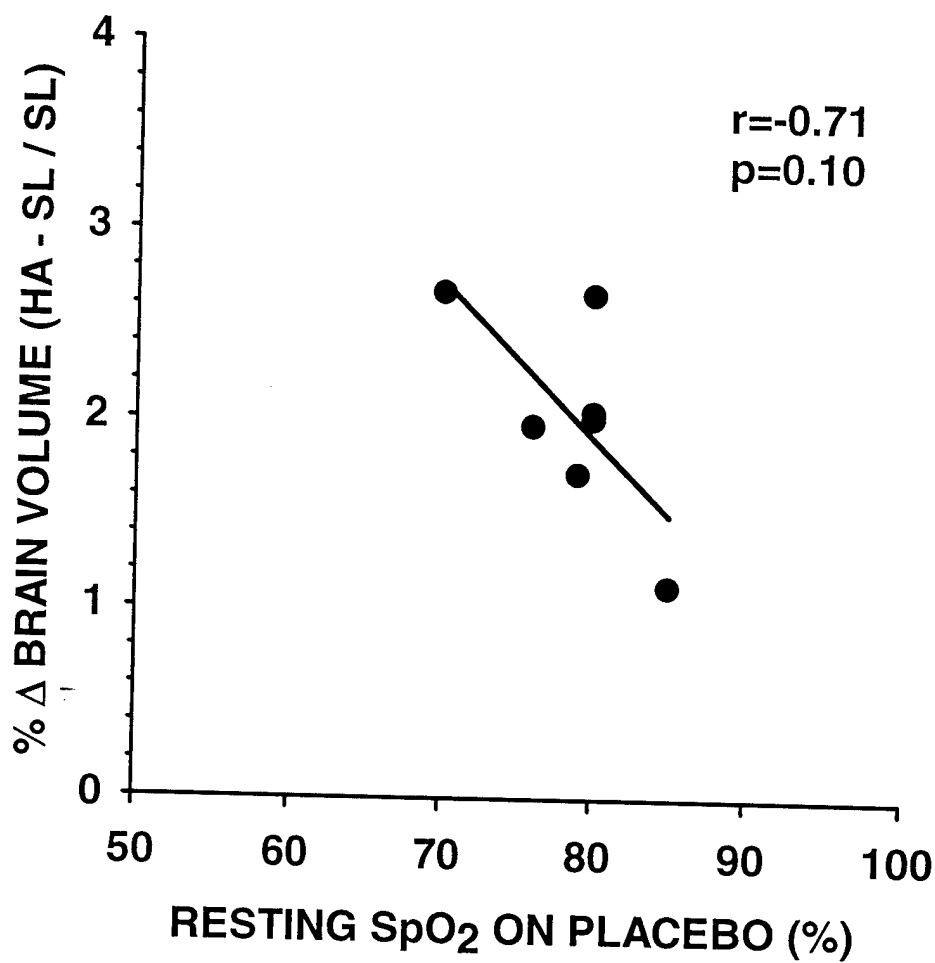


FIGURE 6
Magnitude of brain volume increase categorized
by Incidence of AMS during placebo trial

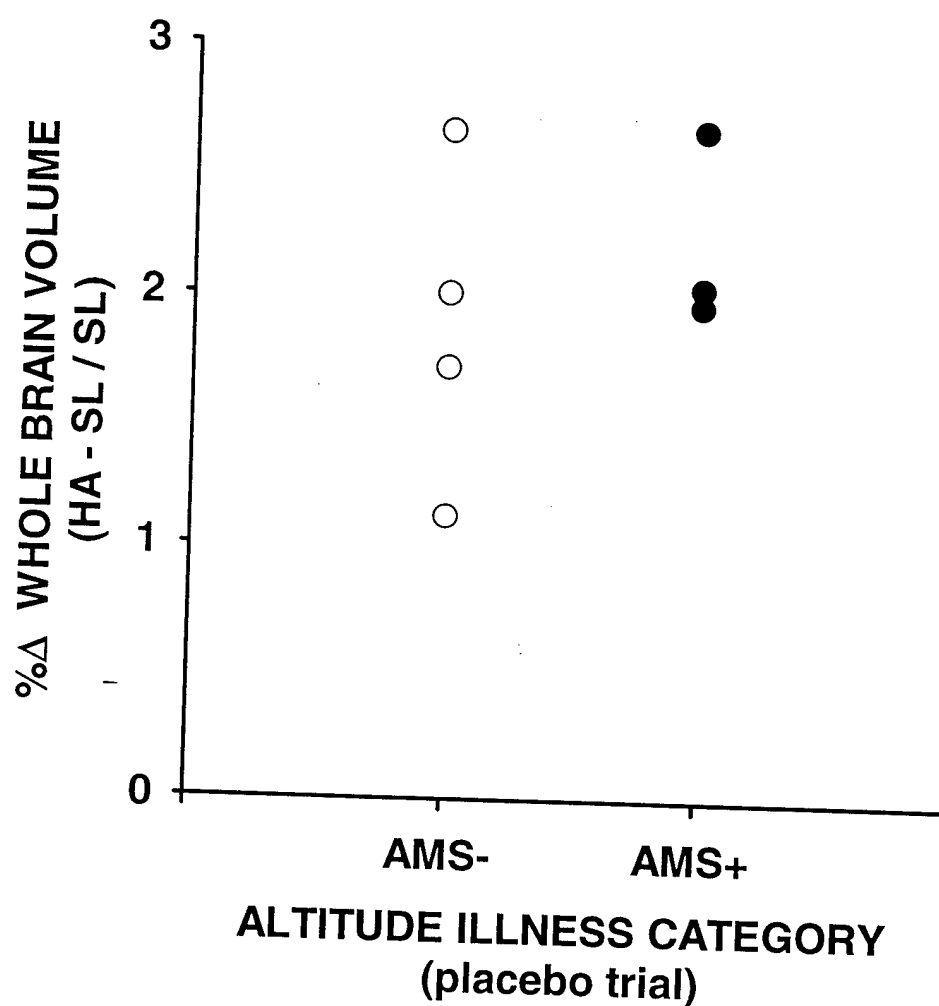
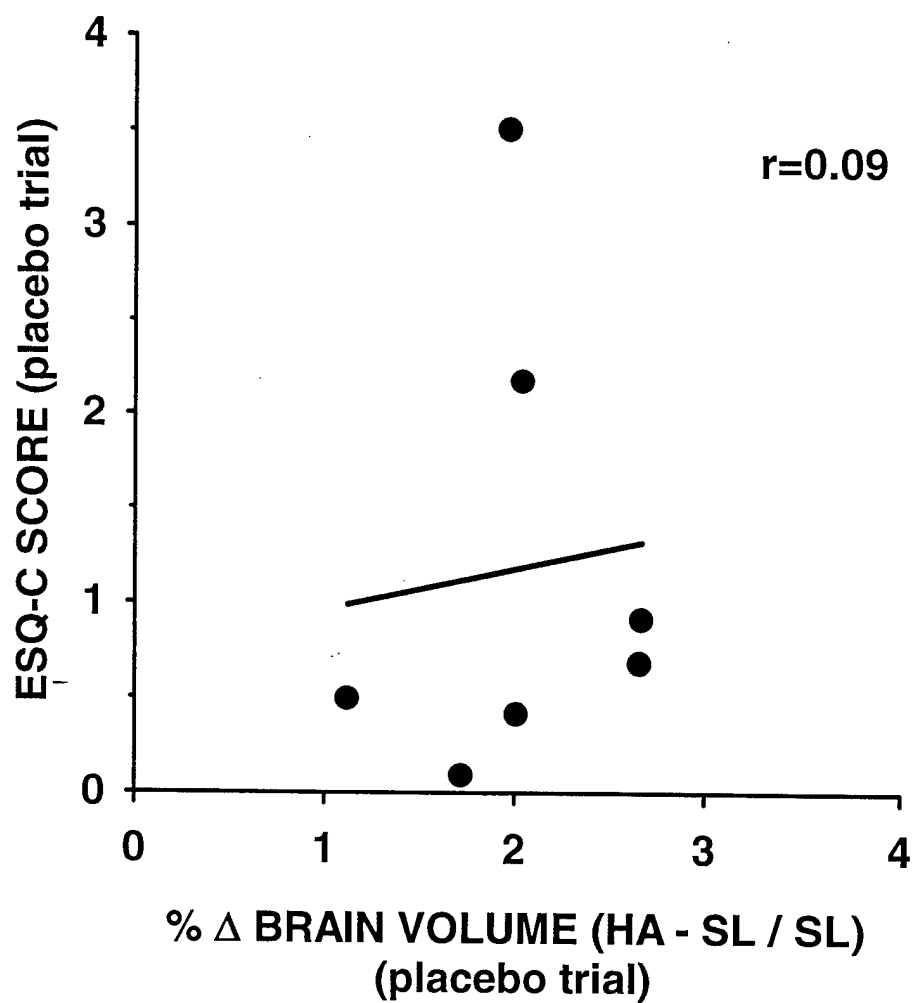


FIGURE 7
Severity of the AMS cerebral symptoms plotted
as a function of the magnitude of brain volume increase



DISCUSSION

This study tested the hypothesis that ingestion of glycerol prior to and during exposure to high altitude would decrease the prevalence and severity of AMS. The basis for the hypothesis was that the osmotic activity of glycerol would create an osmotic gradient between the blood and the cerebral spinal fluid producing a net movement of water out of the CNS, thus attenuating development of cerebral edema. To assess the effects of oral glycerol on development of cerebral edema, we measured brain volume, which we believed would increase in the presence of cerebral edema, using an MRI technique. Our results do not support the putative therapeutic value of glycerol as a prophylaxis for AMS. We did find a significant increase in brain volume, consistent with mild diffuse cerebral edema, following hypobaric exposure.

In order to test a prophylactic treatment for AMS, it is necessary to produce a high prevalence of AMS within the subject population. Based on previous reports (Hultgren, 1992; Robinson, King, and Aoki, 1971; Johnson and Rock, 1988), the choice of rapid ascent to 4,572 m was designed to produce AMS in greater than 80% of our subjects. At this altitude, we expected rapid onset of AMS symptoms in the placebo trial so we could assess whether the treatment would delay or prevent the onset of AMS. Finally, we sought a wide range of AMS severity to assess whether the treatment decreased the severity of AMS. Analysis of our placebo trial data showed that although we obtained only a 50% prevalence of AMS, we did achieve the desired goals of rapid onset and range of symptom severity among the subjects.

When compared to the placebo trial, treatment with glycerol tended to increase AMS prevalence and severity (Figures 3-4). The time course for development of AMS was similar between the treatment and placebo trials. Thus, the results do not support oral glycerol consumption as a prophylaxis for AMS.

Most of the subjects were unable to consume the required dose of glycerol that was designed to produce a specific osmotic gradient between the brain and blood. The subjects complained of gastrointestinal distress (nausea, vomiting) and that the glycerol solution did not taste good. The increase in symptoms associated with gastrointestinal

distress reported on the ESQ supported this observation (Table 3). Three previous studies (Tourtellotte, Reinglass, and Newkirk, 1972; Murray, Eddy, Paul, Seifert, and Halaby, 1991; 36) have reported nausea and headache from glycerol consumption in studies evaluating hyperhydration to prevent heat injury. However, the prevalence and severity of these adverse reactions were not reported. On the other hand, several studies (Riedesel, Allen, Peake, and Al-Qattan, 1987; Montner, Stark, Riedesel, Murata, Robergs, Timms et al., 1996; Lyons, Riedesel, Meuli, and Chick, 1990; Freund, Montain, Young, Sawka, Deluca, Pandolf et al., 1995) make no mention of whether their subjects experienced any adverse reactions to glycerol ingestion. Thus, it appears that the prevalence of nausea and headache from glycerol consumption in hyperhydration studies is low, but not uncommon.

TABLE 3. Average ESQ Scores for gastrointestinal symptoms questions (Q_#) over 32 hours at high altitude.

Symptoms	Placebo Trial	Glycerol Trial
Gastrointestinal(Q17+23+24+25+26+52)	0.34	0.78

Compared to the reports in the hyperhydration literature, the apparently higher prevalence of adverse reactions to glycerol ingestion in our study may be attributable to the absence of hyperhydration with the glycerol consumption. In our study, the lack of increased fluid intake with the glycerol may have created a higher concentration of glycerol in the gut than present in the previous hyperhydration studies. We speculate that the high glycerol concentration produced the adverse gastrointestinal symptoms. Concurrently, the exposure to hypobaric hypoxia may have decreased the tolerance to glycerol ingestion, or vis-a-vis, the glycerol may have increased susceptibility to AMS. In any case, the results of this study do not support a therapeutic effect of glycerol as a prophylaxis for AMS.

To assess whether AMS was associated with development of cerebral edema, we used a new technique (Kapur et al., 1995; Wells, Grimson, Kikinis, and Jolesz, 1996; Matsumae, Kikinis, Morocz, Lorenzo, Sandor, Albert et al., 1996; Matsumae, Kikinis,

Morocz, Lorenzo, Albert, Black et al.,1996) of 3-dimensional segmentation of volume magnetic resonance imaging data to quantify changes in brain tissue volume. We hypothesized brain tissue volume would increase in the presence of cerebral edema. We found a small (~2.2%), but highly statistically significant increase in whole brain volume following ~32 h hypobaric exposure. With the exception of 1 subject during the glycerol trial, whole brain volume increased in all subjects at high altitude, whether on placebo or glycerol. Glycerol consumption had no effect on the magnitude of the brain volume increase.

The magnitude of the brain volume increase tended to be inversely related to the resting arterial oxygen saturation. If the increased brain volume resulted from cerebral edema, then this finding is consistent with previous reports that AMS prevalence and severity increases as the arterial oxygen saturation decreases (Sutton, Bryan, Gray, Horton, Rebuck, Woodley et al.,1976; Hackett, Roach, Wood, Foutch, Meehan, Rennie et al.,1988; Hackett, Rennie, Hofmeister, Grover, Grover, and Reeves,1982; Hartig and Hackett,1992). However, we did not find any relationship between the presence or severity of AMS and the magnitude of the brain volume increase (Figures 8 and 9). Subjects apparently free of AMS had increases in brain volume of similar magnitude to those subjects reporting AMS symptoms. In fact, in one subject brain volume decreased ~0.5%, yet that subject had an AMS severity score of 3 indicating a notable degree of illness. However, that severity score may have been increased due to the consumption of glycerol, as previously mentioned. Still, the smallest brain volume increase following both hypobaric exposures was observed in the one subject who did not develop AMS during either hypobaric study. The lack of correlation between the measured brain volume changes and prevalence and severity of AMS may be an artifact of the experimental design, since the MRI studies did not coincide with the period of peak AMS symptomatology, in all subjects. Nevertheless, the results indicate that even in the absence of AMS, an increase in brain volume is a likely occurrence during high altitude exposure of the magnitude used in this study.

Our results found that the increase in brain volume was almost entirely within the gray matter. This is at odds with previous reports which suggest edema in the white matter (Matsuzawa et al.,1992; Levine, Yoshimura, Kobayashi, Fukushima, Shibamoto, and Ueda,1989). However, given the reproducibility of this response, we believe our

segmentation of the brain volume changes between the gray and white matter to be accurate. That the gray matter was the site of brain volume increase should not be unexpected given the greater blood flow and capillary density in gray matter than white matter (Stubbs,1983). Presumably, if extravasating fluid from cerebral capillaries is the source of the cerebral edema, then its appearance in the gray matter initially is plausible.

In summary, this study found that oral glycerol consumption did not decrease the prevalence or severity of AMS in young healthy men during an exposure to 4,572 m altitude in a hypobaric chamber. To the contrary, ingesting the glycerol solution may have produced symptoms mimicking AMS and/or accentuated the AMS symptoms. The use of MRI segmentation results to examine changes in brain tissue volume following a 32-h high altitude exposure revealed a reproducible increase in brain volume, primarily of the gray matter, consistent with development of cerebral edema. However, there was no apparent relationship between the AMS prevalence or severity and the magnitude of the brain volume changes.

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